From the
INTERNATIONAL SEARCHING AUTHORITY

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/009067	12.08.2004	13.08.2003

International Patent Classification (IPC) or both national classification and IPC
C12N15/63, C12N15/21, C07K14/56

Applicant
SANDOZ AG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☒ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/009067

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority for the purposes of search.

3. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. II Priority

1. ☐ The following document has not been furnished:

   ☐ copy of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(a)).

   ☐ translation of the earlier application whose priority has been claimed (Rule 43*bis*.1 and 66.7(b)).

   Consequently it has not been possible to consider the validity of the priority claim. This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43*bis*.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.

3. ☒ It has not been possible to consider the validity of the priority claim because a copy of the priority document was not available to the ISA at the time that the search was conducted (Rule 17.1). This opinion has nevertheless been established on the assumption that the relevant date is the claimed priority date.

4. Additional observations, if necessary: ISA o.k.
   Priority CD beim
   Amt eingegang
   tel. EPO NL 20.5.05 mit Prüfg c.s. 1

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-41
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-41
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-41
	No:	Claims	

2. Citations and explanations

   see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1. Reference is made to the following documents:

D1: Biochimie, Vol. 72, 1990, pages 407-415
D2: Journal of Bacteriology, Vol. 163, No. 3, 1985, pages 1222-1228

SECTION V

2 Novelty (**Article 33(2) PCT**)

The subject-matter of the present application does not appear to be disclosed in the prior art as defined in the regulations (**Rule 64 (1)-(3) PCT**).
Therefore, in view of such prior art the subject-matter of the present application (**claims 1-41**) has to be regarded as being new (**Article 33(2) PCT**).

3 Inventive Step (**Article 33(3) PCT**)

The **closest prior art** to evaluate the inventiveness of the present application (**claims 1-41**) is document **D1**, which discloses the periplasmic expression of an heterologous protein (ferredoxin) in a prokaryotic microorganism (*Escherichia coli*). The periplasmic expression is achieved by the use of an expression vector which contains the OmpA signal peptide.

Starting from **D1**, the underlying **technical problem** to be solved by the present application can be considered to lie in the provision of an alternative way to direct the expression of heterologous proteins into the periplasm of the host cell.

The **solution** provided by the Applicant to solve the above problem lies in the use of an expression vector which comprises the signal peptide of the *gac* (glutaryl 7-aminocephalosporanic acid acylase) gene of *Pseudomonas diminuta*.

Document **D2** discloses the molecular cloning and structure of the gene for glutaryl 7-aminocephalosporanic acid acylase from a *Pseudomonas* strain. Although D2 suggest that this enzyme appears to be periplasmic in *Pseudomonas*, it does not provide any clear indication that this is indeed the case nor any experiments that would prove that

the acylase activity is found in the periplasm in *E. coli*.

There is no indication in the prior art that would teach the person skilled in the art to select the signal peptide of the *gac* gene among the large number of periplasmic genes known in the art. In particular, in view of D2, which does not provide a reasonable expectation of success, the person skilled in the art would not consider the signal peptide of the *gac* gene to secrete foreign proteins into the periplasm.
None of the available prior art documents, including D2, suggests the use of the signal peptide of the *gac* gene to direct the expression of a heterologous protein into the periplasm of the host cell.
The *gac* expression system of the present invention is particular suitable for use in a process for the efficient and direct production of mature recombinant proteins of interest in pure form in a high yield.

Therefore, the subject-matter of the present application (**claims 1-41**) is considered to involve an inventive step (**Article 33(2) PCT**).

SECTION VIII

4. Clarity (**Article 6 PCT**)

Claim 13 is directed to a vector but it depends on claim 10, which is directed to a host cell. This renders claim 13 unclear. It appears that claim 13 should rather be drafted as "the host cell according to claim 10".

The same objection applies to **claim 14**.